Rapid Communication

Potential for High Hydrostatic Pressure Processing to Control Quarantine Insects in Fruit

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J. Econ. Entomol. 100(5): 1499–1503 (2007)

ABSTRACT Tests were conducted to determine the potential for high hydrostatic pressure (HPP) to control codling moth, *Cydia pomonella* (L.), and western cherry fruit fly, *Rhagoletis indifferens* Curran. Apples (*Malus* spp.) with codling moth larvae or eggs were treated at 24 and 72 h, respectively, after infestation at a series of pressures between 14,000 and 26,000 pounds per inch^2 (psi). Survivorship was determined the next day for larvae and after 10 d for eggs. Codling moth eggs were more tolerant of HPP treatment than larvae. Mortality of larvae was 97% at 22,000 psi, whereas mortality of eggs at this dose was 29% and not significantly different from the untreated controls. In a second study, no codling moth eggs hatched at any high pressure treatment between 30,000 and 80,000 psi, indicating these pressures were lethal. Various stages of western cherry fruit fly were treated at pressures from 10,000 to 45,000 psi, and survivorship was determined after 24 h. Eggs and third instars were more tolerant of HPP than the first and second instars. Mortality was 100% in western cherry fruit fly eggs and larvae at pressures ≥25,000 psi. Apple and sweet cherry quality after high pressure treatment was poor, but high pressure may have applications to control quarantine pests in other fruits.

KEY WORDS codling moth, *Cydia pomonella*, western cherry fruit fly, *Rhagoletis indifferens*, quarantine treatment

High pressure processing (HPP) (also called high hydrostatic pressure) is used to control food-borne pathogens and spoilage organisms in a variety of processed foods (Torres and Velazquez 2005), and the use of HPP to extend shelf life in value-added fruits and vegetables is increasing. HPP at 85,000–90,000 pounds per inch^2 (psi) is standard to control food-borne pathogens and spoilage organisms in commercial products. HPP has potential as a phytosanitary treatment to control quarantine insect pests in fresh or minimally processed fruits and vegetables. HPP research with insects has been limited (Butz and Tauscher 1995).

Codling moth, *Cydia pomonella* (L.), is a pest of pome and stone fruits and nuts, and a quarantine pest in commodities exported from the United States to Asia. Western cherry fruit fly, *Rhagoletis indifferens* Curran, is a key pest in all the cherry-growing regions of the Pacific Northwest, and it is a quarantine pest of cherries shipped to California and many foreign countries. We conducted tests to determine the potential for high hydrostatic pressure to control codling moth and western cherry fruit fly.

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Materials and Methods

High Pressure Treatment. High pressure treatment was carried out at Avure Technologies (Kent, WA) by using the QUINTUS Food Press (QFP 35L-600). During preliminary tests of the effects of HPP on fruit quality, it was determined that apples (*Malus* spp.) withstood treatment better in sealed bags, whereas cherries (*Prunus* spp.) could be treated free in the water. Therefore, plastic pouches with apples or cherries in nylon organdy sleeves with insects were loaded into a cylindrical basket and hoisted into the treatment vessel. The water-filled vessel is pressurized with the 7XS-6000 intensifier pump. Temperature of the water in the open vessel was maintained at 16–18°C. HPP transmits hydrostatic pressure through the package to the food. Final pressures were achieved within 60 s and held for 1 min before decompression. The total time in the pressure tank varied slightly depending on the amount of pressure assigned for each dose. Control apples and cherries that traveled to Kent, WA, also were loaded into the treatment vessel and allowed to remain there for a total of 5 min.

Codling Moth. Dose–response tests were performed with codling moth eggs and larvae, the stages of the moth associated with the fruit. Codling moth was reared in the laboratory by using an artificial diet (Toba and Howell 1991). Insects were reared at 23 ± 2°C, 50% RH, and a photoperiod of 16:8 (L:D) h. Wax coated bags (9 by 15 by 27.5 cm, depth by width by length) containing 250 pairs (500 total) moths were set up 24 h before use. The bag was placed at 2°C for 5 min to facilitate removal of moths. Moths were then placed on organic apples ('Gala' and 'Golden Delicious') that were placed into a plastic box (8.5 by 27 by 37 cm, depth by width by length); the bottom and top was lined with unbleached muslin to prevent oviposition on the plastic box. Females were allowed to oviposit for 24 h at 23 ± 2°C, 50% RH, and a photoperiod of 16:8 (L:D) h. Eggs were laid on the surface of the fruit. After the oviposition period, the fruit with moths were chilled at 2°C for 5 min to facilitate removal from boxes. The fruit with eggs were held under normal rearing conditions for 72 h to develop to mature red ring or early black head stage before testing. Apples were examined under a dissecting microscope to determine location, stage, and number of eggs on each fruit. Each egg was circled with a felt tip marker and the number of eggs per apple was marked and recorded.

Organic apples (Golden Delicious) were used as a host for infestation with codling moth larvae. The fruit were divided into 6.82-kg lots in plastic boxes (41 by 57.5 by 13.2 cm, width by length by depth). Larvae were removed from the artificial diet using soft forceps and placed directly onto fruit. When placed on fruit, larvae bore into the fruit to feed. In total, 200 larvae were applied to the 6.82 kg of mature fruit, which represents an infestation rate of approximately five larvae per fruit. The top of the box was lined with double stick tape to which a length of nylon organdy was adhered. The top was then sealed with the lid to the plastic box. The infested fruit were placed into an environmentally controlled room and held at 23°C, 50–60% RH, and a photoperiod of 16:8 (L:D) h overnight. A group of 100 larvae of the same stage were used to infest 3.41 kg of fruit to serve as untreated controls and held similarly. Before treatment, infested fruit were removed from the boxes and transferred into clean plastic lugs lined with apple packing trays to prevent fruit from rolling during transport. Any insects outside of the fruit were counted and subtracted from the total infested number to obtain the total number of insects actually receiving the treatment.

Apples with larvae and eggs were treated 24 h after infestation at a series of pressures between 14,000 to 26,000 psi. Survivorship was determined the next day for larvae and after 10 d for eggs. In all treatments, apples were packaged in hermetically sealed 1-liter size plastic pouches (Kapak, Sealpak, 2.5 ml in thickness) without vacuum before treatment, and they were removed from the bags immediately after treatment. In a second study, codling moth eggs were treated at pressures from 30,000 to 80,000 psi in 10,000 psi increments and held before counting mortality as described above. Each pressure treatment in each experiment was replicated three times, by using 8–10 fruit infested with codling moths in each replicate.

Western Cherry Fruit Fly. Infested cherries (159.1 kg; 350 lb) were harvested from a 'Bing' cherry tree at a residential property in Pasco, WA, in June 2006. Harvested cherries were divided by weight equally among treatments. The experimental design was to treat cherries at a series of 10 pressure levels with three replicates at each level, arranged in a completely randomized design. There were two sets of untreated controls; one set of controls was left at the laboratory in Wapato, WA, and the other set traveled to Kent with the cherries to be treated. The treatments were as follows: 0-lab (control left at laboratory); 0 (control dunked in high pressure tank); and 10,000, 15,000, 20,000, 25,000, 30,000, 35,000, 40,000, and 45,000 psi. Approximately 5.3 kg (11.6 lb) of infested cherries was used for each replicate at each treatment level. The infested cherries were placed into 59 by 32 cm (length by width) nylon organdy bags and transported in picking lugs (18 by 35.5 by 55.5 cm, height by width by length) to Kent for treatment. Cherries were not sealed in plastic bags like the apples. They were allowed to float free in the nylon organdy bag during treatment. The cherries contained a range of western cherry fruit fly life stages. The number of individuals of each stage at the time of treatment was determined by recording the date of pupation and extrapolating backwards to the stage present at the time of treatment.

Determination of Larval Mortality. Control and treated apples containing codling moth larvae were held at 22 ± 2°C, 60% RH, and a photoperiod of 16:8 (L:D) h for 24 h before examination of surviving and dead larvae. Fruit were cut with a paring knife and examined for live, dead, and moribund larvae. Moribund larvae were held to 7 d at 22 ± 2°C, 60% RH, and a photoperiod of 16:8 (L:D) h on thinning apples, and then they were assessed for survivorship.

Control and treated sweet cherries containing western cherry fruit fly were placed into 49.5 by 36.0 by 12.4 cm (length by width by depth) plastic containers (Rubbermaid, Wooster, OH) that had wire racks placed in the bottom. The wire racks were 4.5 cm from the bottom of the container. Water was added to the bottom of each container, ≈2 cm, so that there was a medium to catch the larvae and pupae that might drop from the fruit. All the plastic containers were covered with a sheet of nylon organdy that was secured by double-sided tape and placed on two metal racks for the month-long holding period. The cherries were monitored from 6 June to 17 July 2006. Each weekday, the cherries were checked, and any larvae or pupae dead or alive were recorded. More water was added to each plastic container as needed.

Determination of Egg Hatch. Apples with codling moth eggs were placed on layers of paper towels lining plastic containers. The containers containing infested apples were placed at 22 ± 2°C, 60% RH, and a photoperiod of 16:8 (L:D) h for 7–10 d to allow for egg hatch. After the holding period, apples were examined under a dissecting microscope to determine presence or absence of eggs and hatch of eggs.

Fruit Quality. Quality apples were obtained from Inland-Joseph Inc. in Wapato, WA. Three boxes of Washington extra fancy Golden Delicious apples were used to obtain information on product quality after HPP treatments. Fruit were treated separately from

Table 1. Effect of high hydrostatic pressure treatments against codling moth larvae in apples

Pressure (psi)	Total treated	No. apples	Mean mortality (± SEM)
0	64	20	67.6 (14.9)
14,000	91	22	76.8 (4.9)
18,000	106	25	91.5 (1.7)
22,000	84	20	97.7 (2.5)

infestation trials, but they were handled in the same manner as described. Fruit were examined for visible external damage and flesh firmness was determined by using a hand-held penetrometer with a 5-mm tip at 24 h after treatment.

Separate batches of cherries were used to assess fruit quality after high pressure treatment. Cherries were obtained from a locally owned private orchard in Benton City, WA. Two 9.1-kg (20-lb.) boxes were purchased, and the cherries were divided into lots so each of the 10 doses had the same amount of cherries by weight. The doses for the quality tests were the same as described above for the infested cherries. The cherries were placed in nylon organdy bags with the same dimensions as the infested cherries and treated at the same pressure levels (0–45,000 psi).

Statistical Analysis. Codling moth and western cherry fruit fly egg and larval mortality or survival data were subjected to linear regression by using the standard least squares model (SAS Institute 2002). Data used in the linear regression model included any treatment dose causing mortality between 0 and 100%, and the lowest dose causing 100% mortality. Residual plots were evaluated to ensure regression model assumptions were met for each treatment combination. Barotolerance of western cherry fruit fly eggs and third instars was compared using covariance analysis. Data used in the linear regression model included any treatment dose causing mortality between 0 and 100%, and the lowest dose causing 100% mortality. For each replicate, mortality values <100% were adjusted for control mortality using Abbott's formula (Abbott 1925). Percentage of survival data were arcsine transformed to help normalize the distribution. Covariance analysis requires the slopes of the regression lines fitted to each group to be parallel, so the assumption of parallelism (nonsignificant treatment × dose interaction effect) was tested before comparing intercepts (treatment effects) (Sokal and Rohlf 1981).

Results

Codling Moth. In the first experiment, mortality of codling moth larvae increased significantly with increasing pressure ($F_{1, 59} = 9.0; P < 0.01$) (Table 1). The equation for the regression line by using untransformed percentage mortality data were y = 66.0 + 0.0015 (pressure level [psi]) ($R^2 = 0.13$). After correcting for control mortality, the 22,000-psi treatment killed 97.1% of the larvae. Larval mortality for the 26,000-psi treatment was not determined, because many larvae drowned in the badly damaged apples.

Table 2. Effect of high hydrostatic pressure treatments against codling moth eggs on apples in the first (top) and second (bottom) tests

Pressure (psi)	No. apples	No. eggs	No. intact eggs	Mean % egg hatch (± SEM)
0 (control)	5	332	295	39.3 (11.7)
14,000	6	407	328	40.2 (7.8)
18,000	3	287	244	27.1 (7.2)
22,000	4	322	289	28.8 (6.0)
26,000	4	370	349	33.6 (10.5)
0 (control)	7	555	309	52.7 (7.4)
30,000	6	632	11.3	0.0
40,000	6	646	9.6	0.0
50,000	6	576	15.0	0.0
60,000	6	583	13.2	0.0
70,000	6	646	10.3	0.0
80,000	6	726	12.1	0.0

Egg hatch was unaffected by high pressure treatment between 14,000 and 26,000 psi (Table 2). The equation for the regression line by using untransformed percentage of hatch data were y = 59.7–0.00036 (pressure level [psi]) ($R^2 = 0.03$), and the slope was not significantly different from 0 ($F_{1, 20} = 0.67; P < 0.42$). Egg hatch in the control treatment was not significantly different from any pressure treatment ($P < 0.05$; Dunnett's method). The egg stage was more tolerant of pressure than the larval stage over the same range of pressures. In the second experiment using eggs, no eggs hatched in any of the high pressure treatments between 30,000 and 80,000 psi, whereas 52.7% of intact eggs hatched in the control treatment (Table 2). This indicates that pressure treatment at ≥30,000 psi should be effective as a quarantine treatment against codling moth.

Golden Delicious apples treated at doses from 14,000 to 22,000 psi were severely damaged by the treatment. Immediately after treatment, liquid was observed to exude from the lenticels of the fruit. Twenty-four hours after treatment, treated fruit were significantly softer than control fruit, and the surface of the fruit seemed to be translucent and highly bruised. Average penetrometer readings of controls was 6 psi, whereas penetrometer readings of treated fruit were near 1 psi. Any attempt to further assess fruit quality was abandoned after visual assessments.

Western Cherry Fruit Fly. For western cherry fruit fly eggs and third instars, survival decreased significantly with increasing pressure ($F_{1, 10} = 12.2; P < 0.01$ and $F_{1, 10} = 13.1; P < 0.01$, respectively) (Table 3). The equation for the regression line using untransformed

Table 3. Percentage of survival (means ± SEM) of western cherry fruit fly immature stages treated with high hydrostatic pressure

Dose (psi)	Stage			
	Egg	First instar	Second instar	Third instar
10,000	9.86 ± 3.72	4.85 ± 4.85	0.00 ± 0.00	6.64 ± 1.40
15,000	7.04 ± 1.41	0.00 ± 0.00	0.00 ± 0.00	7.69 ± 2.45
20,000	5.64 ± 1.01	0.00 ± 0.00	0.00 ± 0.00	0.35 ± 0.35
25,000	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

percentage of survival data were y = 16.5–0.00062 (pressure level [psi]) (R^2 = 0.55) for eggs and y = 13.2–0.00055 (pressure level [psi]) (R^2 = 0.57) for third instars. No second instars survived any of the pressure treatments, and first instar survivors occurred only at the lowest pressure treatment (Table 3). Western cherry fruit fly egg and third instars were compared using covariance analysis on arcsine-transformed data. The stage by dose interaction effects were not significant ($F_{1, 2} = 0.04$; $P = 0.85$), suggesting slopes were parallel and therefore intercepts (stage effects) could be compared. The egg and third instar stages were not significantly different ($F_{1, 2} = 3.5$; $P = 0.08$), but the trend was for higher survival in eggs (Table 3). No western cherry fruit flies survived at 25,000 psi, suggesting this might be an effective quarantine treatment against this insect.

Sweet cherry quality directly after treatment did not seem to be affected. However, 24 h after treatment, fruit became increasingly softer with increasing doses of hydrostatic pressure. In addition, browning of stems increased with increasing dose, and stems in all HPP treatments were considerably worse than controls. The red 'Bing' fruit looked almost translucent directly after treatment and then turned brown or mahogany in color, deepening with increasing dose of hydrostatic pressure. Alternative treatment conditions to avoid quality problems such as treatment at cold temperatures (0–4°C) were not tested.

Discussion

High hydrostatic pressures ≥30,000 psi controlled all stages of both codling moth and western cherry fruit fly. The eggs of codling moth were more tolerant of pressure than the larval stages. The eggs and third instars of Western cherry fruit fly were equally tolerant of pressure and more tolerant than the other larval stages tested. Fruit quality was not acceptable for either apples or sweet cherries based on visual assessment and firmness values. However, this HPP effectively controlled the two quarantine insects, and it may have potential to control other pests in commodities that can tolerate these high pressures, such as avocados, *Persea americana* Mill., and mangoes, *Mangifera indica* L. (E.R., unpublished data).

Codling moth and western cherry fruit fly seem to be more tolerant of HPP than Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Whereas Mediterranean fruit fly eggs were completely inactivated at ≈18,000 psi (Butz and Tauscher 1995), this pressure showed no increased mortality over untreated controls in codling moth eggs (Table 2). Western cherry fruit fly eggs showed ≈5% survival at 20,000 psi.

The physiological effect of HPP on insects has not been studied. High pressure inactivation of bacteria seems to be multitarget, with the cytoplasmic membrane as a key target and evidence for other damaging events such as extensive solute loss, protein coagulation, and enzyme inactivation and ribosome conformational changes with impaired recovery sometimes contributing to cell death (Manas and Pagan 2005). Multifactorial injury in insects is likely, particularly in the larval, pupal, and adult stages. Baseline hemocoelic pressures in codling moth pupae are ≈200–500 Pa (Slama and Neven 2001). These pressures help maintain closure of the spiracles covering the tracheal system. HPP may override hemocoelic pressures, allowing for the tracheoles to become flooded with water. HPP also may inhibit dorsal aorta beating by inhibiting hemocoelic fluid flow in insects. Without appropriate tissue perfusion and blocked respiratory system, systemic oxygen deprivation will begin to cause cell death. The effects of pressure on the egg stage of insects that lack vulnerable functioning organ systems needs investigation.

HPP effects are uniform and almost instantaneous throughout the product; thus, they are independent of food geometry (Torres and Velazquez 2005). However, HPP can cause unacceptable damage to many fresh fruits and vegetables. In our tests, sweet cherries and apples treated with even low levels of pressure were partially crushed during treatment. Certain fresh fruits such as avocados and mangos tolerate pressure processing without damage, and commercial products with extended shelf life are now in national circulation (Torres and Velazquez 2005). Both these fruits are hosts for tephritid fruit flies and other quarantine pests, and it is unknown whether they remain competent hosts after minimal processing and packaging. HPP may be practical for certain minimally processed foods that are infested with quarantine insects, such as dried fruits and nuts.

HPP adheres to both national and international organic standards (McEvoy 2006, NOPP 2006). High pressure leaves no residues and it is not polluting. HPP treatment at pressures used to inactivate bacteria (85–90,000 psi) seems to be sufficient to control insects, although further research is needed with a wider variety of insects. Scale-up of HPP for commercial application with fresh fruits or vegetables should not pose a problem as such systems are currently in use in the shellfish and processed foods industries (Murchie et al. 2005, Torres and Velazquez 2005).

Acknowledgments

We thank Linda Rehfield-Ray, Dan Hallauer, Kaylin Phillips, Anne Kenny-Chapman, and Megan Vangsted for technical assistance. We also thank Drs. Judy Johnson and Young-Biao Liu for providing peer reviews of this manuscript.

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Received 19 April 2007; accepted 17 June 2007.